intermediates. From the force spectroscopy measurements, we mapped the pathway to the misfolded structure, finding that an intermediate that folds rapidly at high force initiated the misfolding. These results provide insight into the microscopic mechanisms of structural conversion in prion protein misfolding.

858-Pos Board B627
Elucidating Hydrodynamic Force Regulation of Von Willebrand Factor in Hemostasis
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Von Willebrand factor (VWF) is a multimeric plasma glycoprotein that plays a pivotal role in hemostasis - it serves as a ligand for platelet adhesion and aggregation forming a hemostatic plug. The hemostatic potential of VWF is regulated by hydrodynamic forces arising in the circulation. Hydrodynamic shear at sites of bleeding activate the binding of VWF to platelet receptor GpIb. Hydrodynamic forces in blood flow also attenuated thrombosis promoting ultralarge VWF by activating cleavage in the VWF A2 domain by the metalloprotease ADAMTS13. Here, we investigated how mechanical forces, which occur in the circulation, regulate the structural conformation and function of VWF at the molecular level using a combination single-molecule manipulation and computational modeling. We created various hydrodynamic flows to mimic a wide range of blood flow conditions, while directly visualizing the conformational dynamics of single VWF molecules. First, we developed a assay that uses a microfluidic device with a cross-slot geometry to study VWF under elongational flow and developed a custom "shear wheel" microscope to study VWF under shear flow. These techniques will enable us to understand how hydrodynamic forces in the bloodstream act on VWF to cause quaternary unfolding, and how this in turn regulates adhesive activity. Second, we developed a kinetic model of force-induced enzymatic cleavage of A2 by ADAMTS13. Our simulations, using parameters from single-molecule force experiments, predict the known healthy size distribution of VWF. We also found that calcium ions protects health-sized VWF from cleavage, and that A2 mutations related to the heritable bleeding disorder von Willebrand disease (VWD) shift hemostatic potential by destabilizing the domain structure under force. These newly developed experimental techniques and findings will lead to a better understanding of bleeding disorders and help progress the therapeutic development of VWD.

859-Pos Board B628
Mapping Protein Structure Changes with Cysteine Labeling Kinetics Measured by Mass Spectrometry
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Protein unfolding, disassembly, and aggregation due to underlie many diseases, but detailed study of these processes in intact cells has been limited. Cysteine Shotgun labeling utilizes cell-permeable fluorescent dyes to label exposed cysteine residues and was initially applied to study protein structure changes in response to mechanical stress on cells. We have re-purposed the technique to identify protein changes in whole-cell lysates in native versus urea-denaturing conditions and in live cells as a function of time and temperature (20-45 deg-C) and mechanical force from stretching. Labeling rate constants are calculated for any given Cys site by normalizing the protein labeling kinetics to the rapid labeling under denaturing conditions. Proteins can be identified and further analyzed by mass spectrometry to pinpoint specific, susceptible domains involved. A number of proteins contain cys with a wide variety of rate constants. Many of these proteins are cytoskeletal, such as Filamin A and B, Talin 1, and Myosin 9. These proteins contain many cysteine-rich domains and appear especially amenable to studying by this new in-cell technique. Many cysteines are near sites of phosphorylation and disease mutations, creating an important new source of data on the effect of these key sites.

860-Pos Board B629
Resolving Cadherin Catch Bond Formation at the Single Molecule Level
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Cadherins are Ca^{2+}-dependent cell-cell adhesion proteins which play key roles in the formation and maintenance of tissues in multi-cellular organisms. We recently showed that cadherins withstand tensile force by forming catch bonds where their bond lifetimes increase with tensile force. Here we resolve the molecular mechanism of cadherin catch bond formation. Using Steered Molecular Dynamics (SMD) simulations, we show that catch bonds are formed because tensile force re-orients the cadherins such that they lock into a tighter contact. Our simulations also predict that cadherins are unable to lock into a tighter binding conformation if their extracellular regions are made more floppy by titrating Ca^{2+} ions from solution. Using single molecule force measurements with an Atomic Force Microscope (AFM), we confirm that catch bond formation is abolished as Ca^{2+} concentration is reduced. Based on these results we propose a molecular model by which cadherins withstand mechanical stress and strengthen cell-cell junctions.

861-Pos Board B630
Single Molecule Force Spectroscopy Studies on the Hydrophobic Hydration
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Hydrophobic interactions underpin many biological self-assembly processes, including protein folding and assembly, ligand-receptor interaction, and micelle and membrane formation. Recent theoretic work by Lum, Chandler and Weeks highlighted the distinct mechanisms for the hydrophobic hydration of small solutes and larger ones. Small solutes can be incorporated into surrounding water molecules by simple entropy driven fluctuations of water, whereas solvation of larger solutes requires the formation of hydrophobic-hydrophilic interface and is an enthalpy-dominated process. However, experimentally verifying this theory is fraught with difficulties. Here, using atomic force microscopy (AFM) based single-molecule force spectroscopy, we quantitatively determine the contribution of entropy and enthalpy for the hydrophobic hydration of polystyrene nanospheres. Our work is based upon pioneering work done by Li and Walker. However, we present a novel data analysis strategy to obtain the free energy profile for the hydrophobic hydration process from the force-extension curves in a model-free fashion. The entropy and enthalpy for the hydrophobic hydration of the polystyrene sphere are in good agreement with theoretic predictions. We focus on the hydration of the nanospheres instead of the stretched polystyrene chains, which allows direct measurement of the crossover length for the two distinct hydrophobic hydration mechanisms of hydration of small and large particles. Our experimental results show that the crossover length is ~ 1 nm and can be tuned by the solvent conditions. Thus, our experiments directly support the length-dependent hydrophobic hydration theory and provide a novel way for the quantification of the free energy for the hydrophobic hydration. Since hydrophobic interactions occur at different length scales in biological systems, we anticipate that our results will be helpful for the understanding of the distinct effect of hydrophobic interactions in various self-assembly process.

862-Pos Board B631
Nanomechanics of the MPMV Capsid-Protein-Binding RNA Fragment
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The interaction between RNA and binding proteins plays an important role in the structure, stability, function and dynamics of either molecular species. The Mason-Pfizer monkey retrovirus (MPMV) is an ideal model for investigating RNA-protein interactions, because part of the genomic RNA, called the packaging signal, is influenced by the binding of its capsid proteins. To explore the properties of MPMV RNA, we manipulated individual molecules of the packaging-signal sequence by using force-measuring optical tweezers.
The 207-base-long segment of MPMV RNA corresponding to the packaging signal, extended on each side with 1200-base-long indifferent gene segments for mechanical handling, was cloned into a pET28a vector, then expressed in an in vitro transcription system. Complementary DNA strands were hybridized to this RNA in a thermal-ramp protocol so as to obtain RNA/DNA handle ends. The complex was then manipulated in repetitive stretch and relaxation cycles across a force range of 0-80 pN under Mg-free conditions. At a stretch rate of 250 nm/s force increased monotonically and non-linearly. Above 15 pN a two-step transition was typically observed, corresponding to the unfolding of the RNA molecule. The total length gain (~100 nm) associated with the process is in good agreement with the theoretical contour length. The transitions were not reversible during relaxation with a rate of 250 nm/s, suggesting that the force-dependent refolding kinetics are slow in comparison with that of the experiment. However, upon introducing a ~30-second pause before the second mechanical cycle, the unfolding transitions reappeared. Our findings suggest that the packaging signal forms a complex, stable secondary structure that